

## INFLUENCE OF GLUCAGON ON THE SYNTHESIS OF PHOSPHATIDYLCHOLINES AND PHOSPHATIDYLETHANOLAMINES IN MONOLAYER CULTURES OF RAT HEPATOCYTES

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### 1. Introduction

Diacylglycerols function as common precursors in the biosynthesis of triacylglycerols (TG), phosphatidylcholines (PC) and phosphatidylethanolamines (PE) [1]. Several investigators have shown that the distribution of diacylglycerols among TG, PC and PE in rat liver can be influenced by varying the dietary state of the animal [2–4]. Fasting, for example, causes a drastic inhibition in the formation of hepatic TG [2–7] whereas the synthesis of PC and, particularly, that of PE are much less affected [2–4]. These observations suggest that the hormones insulin and glucagon may be involved in the regulation of the biosynthesis of these glycerolipids since the ratio of insulin and glucagon is determined largely by the nutritional state of the animal [8]. In [9] we demonstrated that addition of insulin to monolayer cultures of adult rat hepatocytes [10] does not alter the rate at which exogenous fatty acids are incorporated into TG, PC and PE. Glucagon also has no effect on the formation of PC and PE from exogenous fatty acids but it causes a marked decrease in the rate of TG synthesis from exogenous fatty acids. Experiments with labelled glucose and glycerol as precursors for the glycerol-backbone of the glycerolipids led also to the suggestion that the increased levels of glucagon occurring in the blood during fasting [11], may be important in maintaining the rate of hepatic phospholipid synthesis at the expense of TG synthesis. Several observations [2–4] indicate that the synthesis of PE responds differently to changes in the dietary state than that of PC and that the levels of hepatic PE and PC are differently affected. These

observations prompted us to investigate whether glucagon may also exert specific effects on the synthesis of PC and PE. The results suggest that during fasting glucagon may preferentially channel diacylglycerols into PE possibly by increasing the availability of CDPethanolamine.

### 2. Materials and methods

Male Wistar rats (250–300 g) were meal-fed standard laboratory chow pellets between 4 a.m. and 7 a.m. Animals were sacrificed at 9 a.m. Hepatocytes were isolated by a modification [12] of the method in [13]. The isolated cells were suspended in a medium as in [10] but without insulin. The cells were plated in vented plastic Petri dishes (Falcon, 60 mm) [10]. Full details of the procedures for isolating and culturing of the cells have been reported [14]. After plating for 3 h, the medium was removed by aspiration and 4 ml fresh medium added with or without glucagon (10 mM). After another 3 h [ $^{32}$ P]phosphate (25  $\mu$ Ci, spec. act. 5–20 Ci/g), [ $Me$ - $^{14}$ C]choline (2  $\mu$ Ci, spec. act. 55 Ci/mol) or [1,2- $^{14}$ C<sub>2</sub>]ethanolamine (2  $\mu$ Ci, spec. act. 55 Ci/mol) were added to the incubation medium to measure the formation of labelled PC, PE, phosphocholine, phosphoethanolamine, CDPcholine and CDPethanolamine. After the appropriate incubation periods, the medium was removed by aspiration and the cells were washed 3 times with ice-cold 0.15 M KCl. Subsequently, the cells were removed from the plates [9] and the lipids extracted as in [15]. The water-soluble intermediates phosphoethanolamine and CDPethanolamine, labelled after incubation

with  $[1,2-^{14}\text{C}_2]$ ethanolamine, were extracted [16] and isolated via thin-layer chromatography on silica-H plates using methanol/0.5% (w/v) NaCl/conc.  $\text{NH}_3$  (50:50:5, v/v/v) as developing solvent [16]. Phosphocholine and CDPcholine, labelled after incubation in the presence of  $[\text{Me-}^{14}\text{C}]$ choline, were extracted [16] and isolated by paper chromatography [17]. The analysis of the lipids was performed as reported before [4]. Protein contents were estimated by the Lowry method [18].

### 3. Results and discussion

The effects of glucagon on the synthesis of PC and PE were studied in monolayers of hepatocytes derived from adult, meal-fed rats. There is ample evidence that such preparations exhibit many functional and morphological features of normal adult liver for extended periods of time (see, e.g., [10,14]).

Table 1 shows the rate of incorporation of label from  $^{32}\text{P}_i$ ,  $[\text{Me-}^{14}\text{C}]$ choline and  $[1,2-^{14}\text{C}_2]$ ethanolamine into PC and PE in the presence and absence of glucagon. Preliminary experiments had shown that the reactions proceed at linear rates up to at least 60 min. It is interesting to note that glucagon exerted opposing effects on the synthesis of PC and that of PE when  $^{32}\text{P}_i$  was used as precursor. Whereas glucagon stimulated the uptake of  $^{32}\text{P}_i$  into PE, it decreased the incorporation of this precursor into PC. The entry of  $[\text{Me-}^{14}\text{C}]$ choline into PC showed also a tendency to diminish although this decrease was statistically

not significant. However, the formation of PE from labelled ethanolamine was significantly enhanced by the addition of glucagon corroborating the positive effect of this hormone on the labelling of PE with  $^{32}\text{P}_i$ . The entry of label from  $[1,2-^{14}\text{C}_2]$ ethanolamine into PC, which proceeds via *N*-methylation of PE [19], is also increased in the presence of glucagon. Whether this is due to a direct effect of glucagon on the *N*-methylation process or to the enhanced availability of PE cannot be concluded from the present information. The effects of glucagon on the synthesis of PE may explain observations from dietary studies [2,4] indicating that the rate of synthesis of PE in the liver *in vivo* is less sensitive to fasting than that of PC.

In other experiments (data not shown), the hepatocytes were incubated in the presence and absence of 85 mM insulin. It was found that this hormone did not significantly affect the labelling of PC and PE with radioactive choline and ethanolamine, respectively. In this connection it is important to emphasize that these hepatocytes in monolayers are insulin-sensitive. It has been shown that physiological concentrations of insulin accelerate, for example, the synthesis of fatty acids in these cells [14].

It was thought of interest to investigate the effects of glucagon on the processes involved in the production of CDPcholine and CDPethanolamine since it has been proposed that the supply of these compounds may be one of the factors controlling the rate of synthesis of PC and PE [16]. In separate experiments it was shown that the formations of phosphoethanolamine, CDPethanolamine, phosphocholine

Table 1  
The effects of glucagon on the rate of synthesis of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from  $^{32}\text{P}_i$ ,  $[\text{Me-}^{14}\text{C}]$ choline and  $[1,2-^{14}\text{C}_2]$ ethanolamine in hepatocyte monolayer cultures

| Addition | $^{32}\text{P}_i$          |                           | $[\text{Me-}^{14}\text{C}]$ Choline |                | $[1,2-^{14}\text{C}_2]$ Ethanolamine |                               |
|----------|----------------------------|---------------------------|-------------------------------------|----------------|--------------------------------------|-------------------------------|
|          | PE                         | PC                        | PE                                  | PC             | PE                                   | PC                            |
| None     | 820 $\pm$ 42               | 470 $\pm$ 37              | —                                   | 5623 $\pm$ 598 | 86 583 $\pm$ 4627                    | 10 383 $\pm$ 668              |
| Glucagon | 1053 $\pm$ 53 <sup>a</sup> | 335 $\pm$ 25 <sup>a</sup> | —                                   | 5138 $\pm$ 352 | 104 768 $\pm$ 3763 <sup>a</sup>      | 13 080 $\pm$ 853 <sup>a</sup> |

<sup>a</sup> Different from the control with  $P < 0.05$

The cells were incubated in the presence of the indicated labelled precursors for 60 min. For further experimental details see section 2. Results are expressed as dpm/mg protein.h. Each value represents the average  $\pm$  SD of  $\geq 3$  expt. Each experiment was carried out in 3-fold

Table 2

The effects of glucagon on the rate of synthesis of phosphoethanolamine and CDPethanolamine from  $[1,2-^{14}\text{C}_2]$  ethanolamine and that of phosphocholine and CDPcholine from  $[Me-^{14}\text{C}]$  choline in hepatocyte monolayer cultures

| Addition | $[1,2-^{14}\text{C}_2]$ Ethanolamine |                             | $[Me-^{14}\text{C}]$ Choline   |                |
|----------|--------------------------------------|-----------------------------|--------------------------------|----------------|
|          | Phosphoethanolamine                  | CDPethanolamine             | Phosphocholine                 | CDPcholine     |
| None     | 9976 $\pm$ 293                       | 3666 $\pm$ 486              | 42 045 $\pm$ 4344              | 2370 $\pm$ 656 |
| Glucagon | 18 237 $\pm$ 853 <sup>a</sup>        | 5679 $\pm$ 609 <sup>b</sup> | 55 524 $\pm$ 4429 <sup>c</sup> | 3070 $\pm$ 491 |

<sup>a</sup> Different from the control with: <sup>a</sup>  $P < 0.01$ ; <sup>b</sup>  $P < 0.02$ ; <sup>c</sup>  $P < 0.05$

Results are expressed as dpm/mg protein.h. Each value represents the average  $\pm$  SD of 2 expt. Each experiment was carried out in  $\geq 3$ -fold

and CDPcholine from labelled ethanolamine and choline proceed at linear rates up to at least 30 min. The data of table 2 clearly show that the synthesis of phosphoethanolamine and CDPethanolamine are both accelerated in the presence of glucagon. These results suggest that glucagon exerts its positive effect on the synthesis of PE from  $^{32}\text{P}_i$  and  $[1,2-^{14}\text{C}_2]$ -ethanolamine probably through an enhanced supply of CDPethanolamine. Although glucagon also accelerated the formation of phosphocholine from  $[Me-^{14}\text{C}]$ -choline, it did not significantly affect the synthesis of CDPcholine nor that of PC. This may suggest that the conversion of phosphocholine into CDPcholine represents the rate-limiting step in the formation of PC which is in agreement with the suggestions of other investigators [16,20,21].

There is accruing evidence, largely due to the efforts of Brindley and his colleagues [22], that phosphatidate phosphatase which catalyses the conversion of phosphatidate into diacylglycerols, plays a key-role in the regulation of hepatic lipid metabolism by controlling the flux of diacylglycerols to glycerolipids. However, the different response of the synthesis of hepatic TG, PC and PE to dietary changes already suggested [2–4] that there are additional control mechanisms regulating the partitioning of diacylglycerols among TG, PC and PE. Glucagon may be one of the factors involved in this regulation. Earlier [9] it could be shown that glucagon may preferentially channel diacylglycerols into phospholipids rather than into TG. The present paper shows that this hormone may, in addition, stimulate specifically the synthesis of PE by enhancing the supply of CDP-

ethanolamine. In this context it will be interesting to investigate the effects of glucagon on the activities of the individual enzymes involved in the synthesis of PC and PE.

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